Data Evaluation Report on the Acute Toxicity of DPX-MAT28 (Aminocylopyrachlor Acid) to Fish (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 47560123

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	358148
	OECD Data Point	{.....}
	EPA MRID	47560123
	EPA Guideline	OPPTS 850.1075

Test material: DPX-MAT28 Technical **Purity:** 92.2%
Common name: Aminocyclopyrachlor acid Technical
Chemical name: IUPAC 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS name 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
6-Amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid
CAS No. 858956-08-8
Synonyms DPX-MAT28, Aminocyclopyrachlor

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Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	None	

Date Evaluation Completed: 10/06/09

CITATION: Palmer, S.J., T.Z. Kendall and H.O. Krueger. 2007. DPX-MAT28 Technical: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory study number 112A-215. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study completed December 20, 2007.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to DPX-MAT28 (Aminocyclopyrachlor acid) at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60 and 120 mg ai/L under static conditions; the 96-hour mean-measured concentrations were <0.0210 (<LOD; control), 7.6, 15, 30, 62 and 122 mg ai/L. The 96-h LC_{50} and EC_{50} values were >122 mg ai/L, based on a lack of mortality and sub-lethal effects, yielding a NOAEC value of 122 mg ai/L. Based on the results of this study, aminocylcopyrachlor acid would be classified as practically non-toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

This study is scientifically sound and classified as acceptable. It satisfies the guideline requirement for acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): wet weight: 1.0 (0.67-1.5) g; total length: 4.6 (4.2-5.3) cm.
Measurements were based on the ten negative control fish.
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >122 mg ai/L 95% C.I.: N/A
NOAEC: 122 mg ai/L Probit Slope: N/A
EC_{50}: >122 mg ai/L
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD Guideline for Testing of Chemicals, 203, *Fish Acute Toxicity Test* and U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines, OPPTS Number 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine* (draft). The following deviations from OPPTS 850.1075 were noted:

1. The TOC and particulate matter concentrations in the dilution water were not reported.
2. While one replicate of ten fish per test level is acceptable, guidelines indicate a preference of two replicates per treatment with 10 fish each to improve statistical power by reducing variability unrelated to treatment.

These deviations do not affect the scientific soundness or acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality (United States), Data Confidentiality (countries outside of the United States), GLP and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA Good Laboratory Practice Standards (40CFR parts 160 and 792) that are consistent with the OECD and Japan MAFF Principles of Good Laboratory Practice.

A. MATERIALS:

1. Test material DPX-MAT28 Technical (metabolite of aminocyclopyrachlor)

Description: Solid

Lot No./Batch No. : 009

Purity: 92.2%

Stability of compound under test conditions: Stable. The mean-measured concentrations yielded recoveries of 100-103% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Ambient Conditions

Physicochemical properties of DPX-MAT28.

Parameter	Values	Comments
Water solubility at 20EC	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	

Parameter	Values	Comments
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Juveniles

Weight at study initiation: 1.0 (0.67-1.5)g; based on 10 negative control fish
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: 4.6 (4.2-5.3) cm; based on 10 negative control fish
EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀ 1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout

Source: Thomas Fish Company, Anderson, California
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study authors reported that nominal concentrations were selected in consultation with the study Sponsor and based on results from exploratory range-finding tests; however, no details pertaining to these tests were provided.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	At least 14 days	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Conditions: (same as test or not)	Same as test	
Feeding:	Fish were fed with a commercially-prepared diet (Zeigler Brother, Inc., Gardners, PA). Feeding was terminated at least two days prior to testing.	
Health: (any mortality observed)	No sub-lethal effects or mortalities were observed during the acclimation period.	
Duration of the test	96 hours	

Parameter	Details	Remarks *Criteria*
		The recommended test duration is 96 hours.
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	None	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Stainless steel 54 L 40 L	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Water was obtained from a freshwater well approximately 40 meters deep located on the Wildlife International, Ltd. site. Water was passed through a sand filter, aerated with spray nozzles and then passed through a 0.45 µm filter to remove fine particles before use.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency=s 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf)* *Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	124 mg/L as $CaCO_3$ 7.9-8.5 ≥7.9 mg/L (≥73% of saturation) Not Reported Not Reported See Reviewer's Comments None Detected 4.8 mg/L as chloride 11.9-12.1°C N/A Temperature, DO and pH were measured in each test chamber at test initiation and every 24 hours thereafter. Temperature was also measured continuously in the negative control test chamber.	*Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃ 60% during first 48 hrs and ∃ 40% during second 48 hrs; flow-through: ∃ 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 1 N/A 1	*Recommended number of replicates include a control and five treatment levels with 2 replicates per level. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	*Number of organisms per replicate should be ∃ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.25 g/L	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured:	0 (negative control), 7.5, 15, 30, 60 and 120 mg ai/L <0.0210 (<LOD; control), 7.6, 15, 30, 62 and 122 mg ai/L	
Solvent (type, percentage, if used)	N/A; a solvent was not used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D with a 30 minute transition period of low-light intensity.	Light intensity at test initiation was 406 lux at the surface of the water of one representative test chamber. *The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the definitive test.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization Level of detection	 0 and 96 hours; HPLC 4.00 mg ai/L 0.0210 mg ai/L	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None Reported	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Sub-lethal effects	
Observation intervals	0, 24, 48, 72 and 96 hours	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortalities occurred in the control or treatment groups throughout the test yielding NOAEC and LC_{50} values of 122 and >122 mg ai/L, respectively.

Table 3: Effect of DPX-MAT28 Technical on Mortality of *Oncorhynchus mykiss*.

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	10	0	0	0	0	0	0
7.6 (7.5)	10	0	0	0	0	0	0
15 (15)	10	0	0	0	0	0	0
30 (30)	10	0	0	0	0	0	0
62 (60)	10	0	0	0	0	0	0
122 (120)	10	0	0	0	0	0	0
NOAEC	122 mg ai/L						
LC_{50}	>122 mg ai/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A- Not applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the negative control or treatment groups throughout the definitive exposure period yielding NOAEC and EC_{50} values of 122 and >122 mg ai/L, respectively.

Table 4: Sub-lethal Effect of DPX-MAT28 Technical on *Oncorhynchus mykiss*.

Mean-Measured and (Nominal) Concentrations mg ai/L	Observation Period		
	Observations- Day 1	Observations- Day 3	Observations- Day 4
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
7.6 (7.5)	A.N.	A.N.	A.N.
15 (15)	A.N.	A.N.	A.N.
30 (30)	A.N.	A.N.	A.N.
62 (60)	A.N.	A.N.	A.N.
122 (120)	A.N.	A.N.	A.N.
NOAEC	122 mg ai/L		
LOAEC	122 mg ai/L		
EC_{50}	>122 mg ai/L		
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A

A.N.- All surviving fish appear normal and healthy
N/A- Not applicable

C. REPORTED STATISTICS:

All toxicity values were visually determined due to the lack of mortality and treatment-related effects. All toxicity values were based on the 96-h mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The lack of mortality and sub-lethal effects precluded the statistical analysis of the data. Therefore, all toxicity values were visually determined based on the 96-h mean-measured concentrations.

LC_{50}: >122 mg ai/L 95% C.I.: N/A
EC_{50}: >122 mg ai/L 95% C.I.: N/A
NOAEC: 122 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER=S COMMENTS:

The reviewer's results were identical to those of the study authors.

The results from the periodic screening analysis of the dilution water indicated the presence of the following elements: calcium (34.9 mg/L), chloride (4.8 mg/L), fluoride (1.2 mg/L), magnesium (12.8 mg/L), potassium (7.55 mg/L), sodium (19.0 mg/L).

The in-life portion of the definitive toxicity test was conducted from July 30 to August 3, 2007.

G. CONCLUSIONS:

This study study is scientifically sound and classified as acceptable. Based on the complete lack of mortality and sub-lethal effects, the NOAEC and LC_{50} values were 122 and >122 mg ai/L, respectively. Based on the results of this study, aminocylcopyrachlor acid would be classified as practically non-toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

LC_{50}: >122 mg ai/L	95% C.I.: N/A
EC_{50}: >122 mg ai/L	95% C.I.: N/A
NOAEC: 122 mg ai/L	
Probit Slope: N/A	95% C.I.: N/A

III. REFERENCES:

Organization for Economic Cooperation and Development. 1993. Guideline 203: *Fish, Acute Toxicity Test.* OECD Guideline for Testing of Chemicals. Updated Guideline adopted 17 July 1992.

U.S. Environmental Protection Agency. 1996. OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine.* Series 850- Ecological Effects Test Guidelines (draft).

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.